



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Walter Callen et al.

Art Unit : 1632

Serial No. : 10/034,849

Examiner : Maher M. Haddad

Filed : December 21, 2001

Title : ENZYMES HAVING HIGH TEMPERATURE POLYMERASE ACTIVITY AND METHODS OF USE THEREOF

RECEIVED
OCT 24 2003
TECH CENTER 1600/2900

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay M. Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as a CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

2. I declare that the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art, e.g., screening enzymes, and nucleic acids encoding enzymes, for polymerase activity (e.g., thermostable DNA polymerase activity), was very high. Using the teaching of the specification, one skilled in the art could have selected routine methods known in the art at the time of the invention to express variants of exemplary enzymes of the invention and screen them polymerase activity. One skilled in the art could have used routine protocols known in the art at the time of the invention, including those described in the instant specification, to screen for nucleic acids encoding polypeptides having a percent sequence identity to SEQ ID NO:1, or active fragments thereof, for polymerase activity. The

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Date of Deposit

Signature

Typed or Printed Name of Person Signing Certificate

10/14/03

Jeanne Brown Rice
Jeanne Brown Rice

state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art in making and screening for antibodies that specifically bind to a polypeptide and making and immunogenic fragments was also very high. Using the teaching of the specification, one skilled in the art could have selected routine methods known in the art at the time of the invention to make antibodies and screen them for specific binding to exemplary enzymes of the invention. Using the teaching of the specification, one skilled in the art could have selected routine methods known in the art at the time of the invention to select and/or screen for immunogenic fragments.

3. I declare that it was routine to screen for multiple substitutions or multiple modifications of an enzyme-encoding sequence and predictably achieve positive results, e.g., making and identifying variant polymerases or immunogenic fragments of polymerases of the invention. While the numbers of samples needed to be screened may have been high, the screening procedures were routine and successful results (i.e., finding variant polymerases and immunogenic fragments, and the nucleic acids encoding them) predictable. Furthermore, it would not have required any knowledge or guidance as to which are the specific structural elements, e.g., amino acid residues, that correlate with polymerase activity or immunogenicity to create variants or fragments of the exemplary enzymes of the invention and to make and screen them for polymerase activity or immunogenic activity. Accordingly, it would not have taken undue experimentation to make and use the claimed invention, including the genus of polymerases of the invention used to make the antibodies of the invention, and immunogenic fragments of the genus of polymerases of the invention.

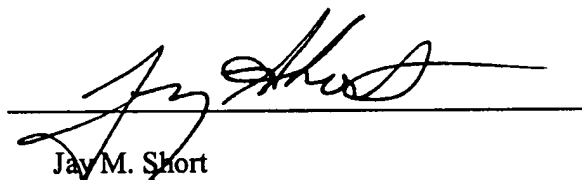
Applicant : Walter Callen et al.
Serial No. : 10/034,849
Filed : December 21, 2001
Page : 3 f3

Attorney's Docket No.: 09010-027006 / DIVER 1350-5

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 10/3/03


Jay M. Short

CURRICULUM VITAE

NAME: Jay M. Short, Ph.D.

EDUCATION:

1981 - 1985	Ph.D., Biochemistry Case Western Reserve University, Cleveland, Ohio
1980 - 1981	Graduate Study, Macromolecular Science Case Western Reserve University, Cleveland, Ohio
1976 - 1980	B.A. with Honors, Chemistry Taylor University, Upland, Indiana

RESEARCH & PROFESSIONAL EXPERIENCE:

<u>1999 - present</u>	CEO, President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
<u>1998 - present</u>	President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
1997 - 1998	Executive Vice President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
1994 - 1997	Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
<u>1990 - 1994</u>	President Stratacyte, Inc. La Jolla, California
<u>1992 - 1994</u>	Vice President R&D (Research) and Operations Stratagene Cloning Systems La Jolla, California
1989 - 1992	Vice President R&D (Research) and Biological Operations Stratagene Cloning Systems
1988 - 1989	Senior Staff Scientist Research and Development Stratagene Cloning Systems

1985 - 1988	Staff Scientist Research and Development Stratagene Cloning Systems
<u>1981 - 1985</u>	Ph.D. Research Case Western Reserve University Dr. Richard W. Hanson's Laboratory, Identification and characterization of the promoter for P-enolpyruvate carboxykinase. First identification of a cAMP regulatory domain.
1980 - 1981	Graduate Student Research Case Western Reserve University Dr. Bruce Roe's Laboratory, Analysis of the cellulase activity of <i>Trichoderma viride</i> .

TEACHING EXPERIENCE:

Thesis Advisor (1988-1993), University of Uppsala, Sweden, Ph.D. for Michelle Alting-Mees
 Lecturer (1992), Committee for Advanced Scientific Education, Center for Drug
 Evaluation and Research, FDA.
 Faculty (1989), Transgenic Mouse Model and Its Application in Assessing
In Vivo Mutagenesis, Genetic Toxicology Workshop (3rd Annual).
 Microbiological Associates Inc. Bethesda, MD.
 Faculty (1987), DNA Cloning and Expression. Physiology Society Workshop. San Diego, CA.
 Teaching Assist., (1981-1985). Molecular and Cellular Biology. Case Western
 Reserve University.
 Teaching Assist., (1981). Physiological Chemistry. Kent State Univ., Kent, OH.
 Teaching Assist., (1978-1980). Quantitative Analysis. Taylor University.

AWARDS, PROFESSIONAL MEMBERSHIPS, ACCOMPLISHMENTS, AND ACTIVITIES:

Visiting Scientist, International Centre of Insect Physiology and Ecology (ICIPE), Kenya (2002-2004)
 Science & Technology Committee, *BIOCOM San Diego*
 Advisory Board, IngleWood Ventures
 Finalists for UCSD Connect's Most Innovative New Product Award in the Biotechnology R&D Category
 Advisory Board, *Chemical & Engineering News*
 Board of Advisors and Founding Member, Division of Biological Sciences, *UCSD*
 Board Director, *BIOCOM San Diego*
 Chairman of the Board, Innovase
 Board Director, Zymetrics
 Board Director, Innovase
 Director at Large, *YPO (Young Presidents' Organization) San Diego*.
 2001 T-Sector Life Science Innovator Award.
 2001 Deloitte and Touche's Orange County / San Diego 2001 Technology "Fast 50".
 San Diego Entrepreneur of the Year 2001.
 YPO (Young Presidents' Organization) San Diego.
 YPO (Young Presidents' Organization) International.
 Finalist for San Diego Entrepreneur of the Year in 2000.
 Largest Biotechnology IPO raising over \$200MM.
 Founding management member of Diversa Corporation.
 Panel for Chemical Science & Technology for NIST, appointed by the National Research Council (1997-2000).
 Chairman (1993), Discussion Group, Society of Toxicology Conference.
 U.S. Committee Member for Evaluation of Biotechnology Research in Spain.
 Editor, Mutation Research.

UCSD Connect Program (1991) 1st Place Award for Innovation and Entrepreneurship in Biotechnology (over 50 competing biotech companies).
 UCSD Connect Program (1990) 1st Place Award for Innovation and Entrepreneurship in Biotechnology.
 Consultant for European Economic Community on Transgenic Toxicology Testing (91-94).
 The New York Academy of Sciences.
 Reviewer for *Proceedings of the National Academy of Sciences*, *Genetic Analysis Techniques*, *Analytical Biochemistry*, & *Nucleic Acids Research*.
 American Association for the Advancement of Science.
 American Chemical Society.
 American Society of Biochemistry and Molecular Biology.
 American Society of Microbiology.
 Environmental Mutagenesis Society.
 Society for Industrial Microbiology
 Society of Toxicology.
 Japanese Environmental Mutagen Society.
 Who's Who Registry of Business Leaders (1994-1995)
 American Men and Women of Science (1995)
 NIEHS Peer Review Committee.
 SBIR Study Section.
 SBIR Annual Report (1993) Program Success Profile (Top 8 of 800 Companies).
 Stratagene (1990) Innovation Award - Lambda ZAP[®] vector.
 Stratagene (1990) Service Award
 Stratagene (1991) Innovation Award - Big Blue[®] Transgenic Testing System.
 Stratagene (1992) Most Innovative Award - Managers/Supervisors.
 Institutional Animal Care and Use Committee (IACUC), Chairman and Institutional Official.
 Award from the University of Victoria for Contributions to the Development of Short-term Transgenic Mutation Assays.
 Nominated as Council Member for the U.S. Environmental Mutagen Society.
 Board Director, *Stressgen (TSE)*, Victoria, BC, Canada
 Board Director & Treasurer, *Stressgen Therapeutics*, Victoria, BC, Canada
 Board Director & Secretary, *Stressgen Therapeutics*, Victoria, BC, Canada
 Board Director, *Diversa*, La Jolla, CA
 Board Director, *Invitrogen*, Carlsbad, CA
 Consultant, *Stratagene Cloning Systems*, La Jolla, CA
 Consultant, *Micro Product Systems*, Lynn, IN
 Reviewer for U.S. Congressional Office of Technology Assessment (OTA) on *The Human Genome Project and Patenting DNA Sequences*.

MEDIA:

ABC Discovery News, ABC San Diego Channel 10, BBC Radio, Bioinformed Newsletter, Biotechnology Newsletter, BioVentures View, Business Daily, Business Week, CEO Cast, Chemical Engineering, Chemical Week, Chemistry & Industry (UK), CNBC, CNN Science & Technology, dBusiness.com, Discovery Magazine, Forbes.com, Good Morning America, Horizon Air Magazine, Idea TV, Inside Business Radio Show, JAG Financial News, Los Angeles Times, NBC San Diego Channel 7/39, National Radio Report, New York Times, Pirateinvestor.com, R&D Magazine, RTL German Television, Reuters, San Diego Business Transcript, San Diego Channel KUSI, San Diego Channel 10, San Diego Magazine, San Diego Union Tribune, Scientist, Time Magazine, The Discovery Channel, The Motley Fool, Time Magazine, USA Today, Wall Street Journal, Wall Street Transcript, Washington Post

PATENTS:

DNA Cloning Vectors with *in vivo* Excisable Plasmids (1987).
 Mutagenesis Testing Using Transgenic Animals Carrying Marker Genes (1987).
 Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test

DNA Sequences (1987).
 Dietary and Hormonal Regulation of Expression of Exogenous Genes in Transgenic Animals Under Control of the Promoter of the Gene Phosphoenolpyruvate Carboxykinase (1988).
 A Transgenic Mouse for Measurement and Characterization of Mutation Induction *In Vivo* (1989).
 Rapid Screening Mutagenesis and Teratogenesis Assay (1989).
 A Combinatorial Approach to Regenerating the Immunoglobulin Repertoire in Prokaryotic Cells (1990).
 Transgenic Animal Models for *In Vivo* Mutagenesis Testing (1990).
 Polycos Vectors (1991).
 A Lambda Packaging Extract Lacking β -Galactosidase Activity (1991).
 A System for Regulation of Eukaryotic Genes (1991).
 Methods for Phenotype Creation from Multiple Gene Populations (1991).
 Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
 Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
 Selectable System Patent (1992).
 Polycos Mutagenesis Systems (1993).
 Use of Trans-acting Proteins for the Development of an *In Situ* Expression Screening System (1993).
 Enzyme Kits and Libraries (1995).
 Enzyme Activity Screening of Clones having DNA from Uncultivated Microorganisms (1995).
 Enzyme Tiered (1995).
 Method for Screening for Enzyme Activity (1995).
 Combined Enzyme Screening/Evolution (1995).
 Uncultured/Activity Screening (1995).
 Directed Evolution of Thermophilic Proteins (1995).
 Combinatorial Enzyme Development (Directed Mutagenesis) (1996).
 Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms (1996).
 Production and Use of Normalized DNA Libraries (1996).
 Methods of DNA Shuffling with Polynucleotides Produced by Blocking or Interrupting a Synthesis or Amplification Process (1996).
 Method of Screening for Enzyme Activity (Biopanning) (1996).
 Directed Evolution of Thermophilic Enzymes (1996).
 Environmental Biopanning (1996).
 Combinatorial Enzyme Development (1996).
 Enzyme Activity Screening of Clones Having DNA from Uncultivated Microorganisms (1996).
 Normalized Samples/Libraries (1996).
 Reassembled Pools of Mutagenized DNA & Procedure (1996).
 Fluorescent-based Single Screening for Enzymes (1996).
 High Throughput Screening for Novel Enzymes (1997).
 Nucleotide Sequence of the *Aquifex aeolicus* Genome, Fragments Thereof, and Uses Thereof (1997).
 Screening for Novel Bioactivities (1997).
 Screening for Novel Compounds which Regulate Biological Interactions (1997).
 Method for Screening Enzyme Activity (1997).
 High Throughput Screening for Novel Enzymes (1997).
 "Discovery" (whole process, including uncultivated, normalized, biopanning, screening, evolving, (etc.) (1997).
 Production of Enzymes Having Desired Activities By Mutagenesis (1999).
 Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms (1999).
 Method of DNA Reassembly by Interrupting Synthesis (1999).
 Production and Use of Normalized DNA Libraries (1999).
 Enzyme Kits and Libraries (1999).
 Screening for Novel Bioactivities (2000).
 Method for Screening for Enzyme Activity (2000).
 Screening for Novel Bioactivities (2000).
 Production and Use of Normalized DNA Libraries (2000).
 Method of Screening for Enzyme Activity (2000).
 Screening Methods for Enzymes and Enzyme Kits (2001).
 Saturation Mutagenesis in Directed Evolution (2001).
 High Throughput Screening for Novel Enzymes (2001).

Recombinant Bacterial Phytases and Uses Thereof (2001).
 Methods Useful for Nucleic Acid Sequencing Using Modified Nucleotides Comprising Phenylboronic Acid (2001).
 End Selection in Directed Evolution (2001)
 Gene Expression Library Produced From DNA From Uncultivated Microorganisms and Method for Making the Same (2001)
 Directed Evolution of Thermophilic Enzymes (2002)
 Method for Screening for Enzyme Activity (2002)
 Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
 End Selection In Directed Evolution (2002)
 Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
 Screening for Novel Bioactivities(2002)
 Method of DNA Shuffling with Polynucleotides Produced or Blocking or Interrupting Synthesis or Amplification Process (2002)
 Production and Use of Normalized DNA Libraries (2002)
 Sequence Based Screening (2002)
 Non-Stochastic Generation of Genetic Vaccines (2002)
 Over 100 Additional Pending Patent Applications Worldwide.

GRANTS AND CONTRACTS:

- *Phase I Small Business Contract #N43-Am-62282. 1985 - 1986. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
- *Phase II Small Business Contract #N43-Am-62282. 1988 - 1990. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
- *Phase I Small Business Grant 2R43ES04484-02. 1986 - 1987. P.I.
Identification of Genetic Lesions Leading to Mutations.
- *Phase II Small Business Grant 2R43ES04484-02. 1989 - 1992. P.I.
Identification of Genetic Lesions Leading to Mutations.
- *1R01-ES04728-01A1. 1989 - 1992. (NIEHS) P.I.
Animal Model for Identification of Genetic Lesions.
- *Phase I Small Business Grant #R43GM42291-01. 1989. P.I.
Switch Mechanism for Gene Expression in Transgenics.
- *RFP NIH-ES-88-11. 1989-1994. (NIEHS) Co-I.
Development of Mutagenesis Assays Using Transgenic Mice.
- *Phase II Small Business Grant #2R44GM42291-02. 1990-1992. (DRG/NIH) P.I.
Switch Mechanism for Gene Expression in Transgenics.
- *Phase I Small Business Grant #1R43GM46585-01. 1991. (DRG/NIH) P.I.
Generation of a Peptide Screening System Through the Development of
Combinatorial-splicing "Polycos" Vectors.
- *Phase I Small Business Grant #1R43CA57066-01. 1992. (NCI) P.I.
Transgenic Rats: A Short-term Mutagenicity Assay for Multi-species Testing of Suspected Human Carcinogens.
- *Phase I Small Business Grant #1R43GM48300-01. 1992. (DRG/NIH) P.I.
Analysis of the Immunoglobulin Hypermutator Mechanism.
- *Phase I Small Business Grant #1R43ES06146-01. 1992. (NIEHS) P.I.
Selectable "Polycos" Shuttle Vectors for In Vivo Mutagenicity Testing.
- *Phase II Small Business Grant #2R44GM46585-02. 1992-1994. (NIGMS) P.I.
Peptide Screening Utilizing Combinatorial Polycos Vector.
- *Phase I Small Business Grant #1R43RR08667-01. 1992-1993. (DRG/NIH) Co-I.
A One-step PCR Cloning System Based on FLP Recombination.
- *Phase II Small Business Grant #2R44CA57066-02. 1993-1995. (NCI) P.I.
Transgenic Rats: Transgenic Rat Model for Mutagenicity Testing.
- *Phase I Small Business Grant. 1993-1994. (NIH) Co-I.
Transgenic Fish Model for Mutagenicity Testing.
- *Phase II Small Business Grant (1994-1996). (NIH) P.I.
"Polycos" Shuttle Vectors for Mutagenicity testing.
- *Phase I Small Business Grant. 1994. (NIH) Co-I.
Vector System for Studying Protein-Protein Interactions.

- *CRADA with LLNL. 1994. (NIH) Co-I.
Mouse and Rat Painting Probes.
- *CRADA with FDA. 1994. (NIH) Co-I.
Tamoxifen Testing in F-344 Rats.
- *CRADA with NASA. 1994. (NIH) Co-I.
Radiation Damage in the Microgravity Environment.

ABSTRACTS AND INVITED LECTURES:

Over 200 Abstracts and Invited Lectures.

PUBLICATIONS:

1. Yoo-Warren, H., Monahan, J.E., Short, J.M., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H.M., Samols, D., and Hanson, R.W. (1983) Isolation and Characterization of the Gene Coding for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) from the Rat. *Proc. Natl. Acad. Sci. U.S.A.*, 80:3656-3660.
2. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1984) Identification of cAMP Regulatory Region in the Gene for Rat Cytosolic Phosphoenolpyruvate Carboxykinase (GTP): Use of Chimeric Genes Transfected into Hepatoma Cells. *J. Biol. Chem.*, 259:12161-12169.
3. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1985) A Region of the Gene for Rat Cytosolic P-enolpyruvate Carboxykinase Confers cAMP Responsiveness to the HSV-thymidine Kinase Gene. In: *Membrane Receptors and Cellular Recognition*, (M. Czech and C.R. Kahn, eds.), Alan Liss Inc., New York, pp 339-346.
4. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. I. Multiple Hormone Regulatory Elements and the Effects of Enhancers. *J. Biol. Chem.*, 261:9714-9720.
5. Short, J.M., Wynshaw-Boris, A., Short, H.P., and Hanson, R. W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. II. Identification of cAMP and Glucocorticoid Regulatory Domains. *J. Biol. Chem.*, 261:9721-9726.
6. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) The Determination of Sequence Requirements for Hormonal Regulation of Gene Expression. *Biotechniques*, 4:104-119.
7. Burns, D.M., Bhandari, G., Short, J.M., Sanders, P.G., Wilson, R.H., and Miller, R.E. (1986) Selection of a Rat Glutamine Synthetase cDNA Clone. *Biochemical and Biophysical Research Communications*, 134:146-151.
8. Hod, Y., Cook, J.S., Weldon, S.L., Short, J.M., Wynshaw-Boris, A., and Hanson, R.W. (1986) Differential Expression of the Genes for the Mitochondrial and Cytosolic Forms of P-enolpyruvate Carboxykinase Gene. In: *Metabolic Regulation: Application of Recombinant DNA Techniques*, (A.G., Goodridge and R.W. Hanson eds.), Annals of the New York Academy of Sciences, New York, Vol. 278, pp. 31-45.
9. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1987) *cis* - acting Regulatory Elements in Hormonally Responsive Genes. In: *Progress in Nucleic Acid Research and Molecular Biology* (W.E. Cohn and K. Moldave eds.), Academic Press, Inc., Orlando, Florida, 34:59-87.
10. Bullock, W., Fernandez, J.M., and Short, J.M. (1987) XL1-Blue: A High Efficiency Plasmid Transforming *recA* *E.coli* Strain With β -Galactosidase Selection. *Biotechniques*, 5:60-64.
11. Short, J.M., Fernandez, J.F., Sorge, J.A., and Huse, W. (1988) Lambda ZAP[®]: A Bacteriophage Lambda Expression Vector With *In Vivo* Excision Properties. *Nucleic Acids Res.*, 16:7583-7600.

12. Short, J.M. (1988) Book Review: Vectors - A Survey of Molecular Cloning Vectors and Their Uses. Raymond L. Rodrigues and David T. Denhardt, eds, Butterworths, Stoneham, MA. *Genomics*, 2:270-271.
13. Short, J.M., and Pollard, A. (1988) Gigapack XL: Size Selective Packaging Extract. *Strategies in Mol. Biol.*, 1:5-7.
14. Kretz, P.L., and Short, J.M. (1989) Gigapack II: A Restriction Deficient (*mcrA*-, *B*-, *hsd*-, *mrr*-) Lambda Packaging Extract. *Strategies in Mol. Biol.*, 2(2):25-26.
15. Kretz, P.L., Reid, C.H., Greener, A., and Short, J.M. (1989) Effect of Lambda Packaging Extract *Mcr* Restriction Activity on DNA Cloning. *Nucleic Acids Res.* 17:5409.
16. Sastry, L., Alting-Mees, M., Huse, W.D., Short, J.M., Sorge, J.A., Hay, B.N., Janda, K.D., Benkovic, S.J., and Lerner, R.A. (1989) Cloning of the Immunological Repertoire in *E. coli* for Generation of Monoclonal Catalytic Antibodies I. Construction of a V_H Specific cDNA Library. *Proc. Natl. Acad. Sci. U.S.A.*, 86:5728-5732.
17. Short, J.M. (1989) The Use of Lambda Phage Shuttle Vectors in Transgenic Mice for Development of a Short Term Mutagenicity Assay. In: *Fifth International Conference on Environmental Mutagens*, Alan Liss, Inc., New York, Part A:335-367. Article and Lecture.
18. Alting-Mees, M., and Short, J.M. (1989) pBluescript II: Gene Mapping Vectors. *Nucleic Acids Res.*, 17:9494.
19. Shopes, B., Alting-Mees, M., Amber, J.R., Ardourel, D., Callahan, M., Detrick, J., Hay, B.N., Hogrefe, H.H., Greener, A., Gross, E.A., Kubitz, M.M., Mullinax, R.L., Wilson, C., Short, J.M., and Sorge, J.A. (1990) Bacteriophage Immuno-expression Libraries: An Emerging Technology for the Identification and Production of Monoclonal Antibodies. *Antibody Engineering, New Tech. & Application Implications*. pp. 98-101.
20. Alting-Mees, M., Amberg, J., Ardourel, D., Elgin, E., Greener, A., Gross, E.A., Kubitz, M., Mullinax, R.L., Short, J.M., and Sorge, J.A. (1990) Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas. *Strategies in Mol. Biol.*, 3:1-9.
21. Kohler, S., Provost, S., Dycaico, M., Sorge, J., and Short, J.M. (1990) Development of a Short-term, *In Vivo* Mutagenesis Assay: The Effects of Methylation on the Recovery of a Lambda Phage Shuttle Vector from Transgenic Mice. *Nucleic Acids Res.*, 18:3007-3013.
22. Kohler, S., Provost, G.S., Kretz, P.L., Fieck, A., and Short, J.M. (1990) An *In Vivo* Assay Using Transgenic Mice to Analyze Spontaneous and Induced Mutations at the Nucleic Acid Level. *Strategies in Mol. Biol.*, 3:19-21.
23. Kretz, P., Kohler, S., and Short, J.M. (1990) The Effect of *E. coli* Minute 98 Locus on DNA Containing Eukaryotic Modifications. *Strategies in Mol. Biol.*, 3:21-22.
24. Mullinax, R.L., Gross, E.A., Amberg, J., Hogrefe, H., Kubitz, M., Greener, A., Alting-Mees, M., Ardourel, D., Hay, B.N., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Identification of Human Antibody Fragment Clones Specific for Tetanus Toxin in a Bacteriophage Lambda Immuno-Expression Library. *Proc. Natl. Acad. Sci. U.S.A.*, 87:8095-8099.
25. Cline, J., Lundberg, K., Nielson, K., Sorge, A., Short, J.M., and Mathur, E.J. (1990) StrataClean Resin: Non-Toxic Protein Extraction. *Strategies in Mol. Biol.*, 4(4):49-51.
26. Mullinax, R.L., Gross, E.A., Amber, J.R., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Human Antibody Clones Isolated From a Bacteriophage Lambda Immunoexpression Library. *Strategies in Mol. Biol.*, 4(4):51-52.
27. Provost, G.S., Kohler, S.W., Fieck, A., Kretz, P.L., Molina, T., and Short, J.M. (1990) Short-term Germ Line and Somatic Cell Mutagenesis Testing With *LacI* Lambda Phage Shuttle Vectors in Transgenic Mice. *Strategies in Mol. Biol.*, 4(4):55-56.

28. Kohler, S.W., Provost, G.S., Kretz, P.L., Fieck, A., Sorge, J.A., and Short, J.M. (1990) The Use of Transgenics Mice for Short Term, *In Vivo* Mutagenicity Testing. *Genetic Analysis Techniques*, 7(8):212-218.
29. Shopes, B., Mullinax, R.L., Amber, J.R., Gross, E.A., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., and Sorge, J.A. (1990) ImmunoZAP[®] Bacteriophage Libraries: A New Technology for the Identification and Expression of Monoclonal Antibodies. *Biotech USA Conference Proceedings*, pp.332-341.
30. Raleigh, E.A., Benner, J., Bloom, F., Braymer, H.D., DeCruz, E., Dharmalingam, K., Heitman, J., Noyer-Weidner, M., Piekarowicz, A., Kretz, P.L., Short, J.M., and Woodcock, D. (1991) Nomenclature Relating to Restriction of Modified DNA in *Escherichia coli*. *Journal of Bacteriology*, 173(8):2707-2709.
31. Kretz, P., Kohler, S., and Short, J.M. (1991) Identification and Characterization of a Gene Responsible for Inhibiting Propagation of Methylated DNA Sequences in *mcrA*, *mcrB1* *E. coli* Strains. *Journal of Bacteriology*, 173:4707-4716.
32. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, B., Sorge, J. A., Putman, D., and Short, J.M. (1991) Spectra of Spontaneous and Induced Mutations Using a Lambda ZAP[®] LacI Shuttle Vector in Transgenic Mice. *Proc. Natl. Acad. Sci. U.S.A.*, 88(18):7958-7962.
33. Wyborski, D., and Short, J.M. (1991) Analysis of Inducers of the *E.coli* Lac Repressor System in Mammalian Cells and Whole Animals. *Nucleic Acids Research*, 19:4647-4653.
34. Lundberg, K.L., Shoemaker, D.D., Adams, M.W.W., Short, J.M., Sorge, J.A., and Mathur, E.J. (1991) High Fidelity Amplification With a Thermostable DNA Polymerase Isolated from *Pyrococcus Furiosus*. *Gene*, 108:1-6.
35. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, W.O., Putman, D.L., Sorge, J.A., and Short, J.M. (1992) Analysis of Spontaneous and Induced Mutations in Transgenic Mice Using a Lambda ZAP[®]/LacI Shuttle Vector. *Environmental and Molecular Mutagenesis*, 18:316-321.
36. Fieck, A., Wyborski, D., and Short, J.M. (1992) Modifications of the *E. coli* Lac Repressor for Expression in Eukaryotic Cells: Effects of Nuclear Signal Sequences on Protein Activity and Nuclear Accumulation. *Nucleic Acids Research*, 20:1785-1791.
37. Hay, B., and Short, J.M. (1992) ExAssistTM Helper Phage and SOLRTM Cells for Lambda ZAP[®] II Excisions. *Strategies in Mol. Biol.*, 5:16-18.
38. Short, J.M. (1992) Tissue Specific Mutagenesis in Transgenic Mice. *The Toxicology Forum, 1992 Annual Winter Meeting*, pp.79-109.
39. Alting-Mees, M.A., Sorge, J.A., and Short, J.M. (1992) pBluescript II: Multifunctional Cloning and Mapping Vectors. *Methods in Enzymology*, 216:483-495.
40. Short, J.M., and Sorge, J.A. (1992) *In Vivo* Excision Properties of Bacteriophage Lambda ZAP[®] Expression Vectors. *Methods in Enzymology*, 216:495-508.
41. Short, J.M. (1992) Transgenic Animals for Carcinogenicity and Genotoxicity Testing. *Biotechnology International, The Global Review of Industry Manufacture and Application 1992*. Section.2. pp. 91-99.
42. Provost, G.S., Hamner, R., Kretz, P.L., and Short, J.M. (1992) Response to the Commentary Article: Comparison of Mutation Frequencies Obtained Using Transgenes and Specific-locus Mutation Systems in Male Mouse Germ Cells. *Mutation Research*, 298:145-147.
43. DuCoeur, L.C., Wyborski, D.L., and Short, J.M. (1992) Control of Gene Expression in Eukaryotic Cells Using the Lac Repressor System. *Strategies in Mol. Biol.*, 5(3):70-72.
44. Jerpseth, B., Greener, A., Short, J.M., Viola, J., and Kretz, P.L. (1992) XL1-Blue MRF: McrA-, McrCB-, Mrr-, HsdRMS- derivative of XL1-Blue. *Strategies in Mol. Biol.*, 5(3):81-83.

45. Altting-Mees, M., Hoener, P., Ardourel, D., Sorge, J., and Short, J.M. (1992) ZAP Express™ and pBK-CMV, pBK-RSV Phagemid Vectors for Prokaryotic and Eukaryotic Expression. *Strategies in Mol. Biol.*, 5(3):58-61.
46. Short, J.M., Provost, G.S., Kretz, P.L., and Dyaico, M.J. (1992) Overview of the Big Blue® *In Vivo* Mutagenesis Assay. *Mammalian Mutagenesis Study Group Communication – JEMS.MMS*, 6:73-89.
47. Lundberg, K.S., Kretz, P.L., Provost, G.S., and Short, J.M. (1993) The Use of Selection in Recovery of Transgenic Targets for Mutation Analysis. *Mutation Research Letters*, 301/2:99-105.
48. Mirsalis, J., Provost, G.S., Matthews, C., Hamner, R., Schindler, J.E., O'Loughlin, K., MacGregor, J.T., and Short, J.M. (1993) Induction of Hepatic Mutations in *LacI* Transgenic Mice. *Mutagenesis*, 8:265-271.
49. Altting-Mees, M.A., Vaillancourt, P., and Short, J.M. (1993) Phagemids and Other Hybrid Vectors. In: *Plasmids: A Practical Approach*. (ed. K. Hardy). IRL Press, pp. 197-223.
50. Provost, G.S., Kretz, P.L., Dyaico, M., Lundberg, K., and Short, J.M. (1993) Transgenic Systems for *In Vivo* Mutation Analysis. *Mutation Research*, 288:133-149.
51. Hedden, V., Callen W., Short, J.M., and Kretz, K. (1993) Improved Sequence Analysis of Mutations Identified With the Big Blue® System. *Strategies in Mol. Biol.*, 6:27-28.
52. Jerpseth, B., Greener, A., Short, J.M., Viola, J., and Kretz, P.L. (1993) New Restriction-Minus Derivatives of XL1-Blue *E. coli* Cells. *Strategies in Mol. Biol.*, 6:24.
53. Vaillancourt, P., Wyborski, D.L., and Short, J.M. (1993) The FLASH® CAT Kit: A Fast, Sensitive CAT Assay Without Radioactivity. *Strategies in Mol. Biol.*, 5:17-19.
54. Short, J.M., Dyaico, M.J., Provost, G.S., Kretz, P.L., Rogers, B.J., Ardourel, D., Wyborski, D.L. and Moofes, J.C. (1993) Transgenic Mice and Rats for Tissue Specific Mutation Analysis. *JEMS*, 22:45-46.
55. Altting-Mees, M.A., and Short, J.M. (1993) Polycos Vectors: Filamentous Phage Packaging Using Lambda Extracts. *Gene*, 137:93-100.
56. Piegorsch, W.W., Lockhart, A.C., Margolin, B.H., Tindall, K.R., Gorelick, N.J., Short, J.M., Carr, G.J., and Shelby, M.D. (1994) Sources of Variability in Data from a *lacI* Transgenic Mouse Mutation Assay. *Environmental & Molecular Mutagenesis*, 23:17-31.
57. Dyaico, M., Provost, G.S., Kretz, P.L., Ransom, S.L., Moores, J.C. and Short, J.M. (1994) The Use of Shuttle Vectors for Mutation Analysis in Transgenic Mice and Rats. *Mutation Research*, 307:461-478.
58. Wyborski, D.L., Malkhosyan, S., Moores, J.C., Dyaico, M.J., and Short, J.M. (1994) Rat2 Cell Line for *In Vitro* Mutagenicity Testing. *Strategies in Mol. Biol.*, 7(2):55-56.
59. Provost, G.S. and Short, J.M. (1994) Characterization of Mutations Induced by Ethylnitrosourea in Seminiferous Tubule Germ Cells of Transgenic B6C3F1 Mice. *Proc. Natl. Acad. Sci. U.S.A.*, 91:6564-6568.
60. Kretz, P.L., Wells, S., and Short, J.M. (1994) Gigapack III: A Single Tube *In Vitro* Lambda Packaging Extract. *Strategies in Molecular Biology* 7:44-45.
61. Knoll, A., Jacobson, D., Kretz, P., Lundberg, K., Short, J., and Sommer, S. (1994) Tissue-Specific Patterns of Spontaneous *LacI* Mutations Recovered From Transgenic Mice. *Mutation Research* 311:57-67.
62. Ashby, J., Short, J.M., Jones, N.J., Lefevre, P.A., Martin, E., Parry, J.M., Burnette, K., Glickman, B.W., and Tinwell, H. (1994) Mutagenicity of o-anisidine to the bladder of *lacI*- transgenic B6C3F1 mice: Absence of ¹⁴C or ³²P bladder DNA adduction. *Carcinogenesis* 15:2291-2296.
63. Altting-Mees, M. and Short, J.M. (1994) Rapid Excision Systems. *Strategies in Molecular Biology* 7(3):70-72.

64. Snead, M., Kretz, P.L., Short, J.M. (1994) Methods for Generating Plant Genomic Libraries. *Plant Molecular Biology Manual* H1:1-19.
65. Rogers, B., Provost, G.S., Young, R., Putman, D.L., and Short, J.M. (1995) Intralaboratory Optimization and Standardization of Mutant Screening Conditions Used for a Lambda/LacI Transgenic Mouse Mutagenesis Assay (I). *Mutation Research* 327:57-66.
66. Young, B., Rogers, B., Provost, G.S., Short, J.M., and Putman, D. (1995) Interlaboratory Comparison of Liver Spontaneous Mutant Frequency from Lambda/LacI Transgenic Mice (Big Blue®) (II). *Mutation Research* 327:67-73.
67. Callahan, J. and Short, J.M. (1995) Transgenic λ /lacI Mutagenicity Assay: Statistical Determination of Sample Size. *Mutation Research* 327:201-208.
68. Wyborski, D.L., Malkhosyan, S., Moores, J.C., Dyaico, M.J., Perucho, M. and Short, J.M. (1995) Development of a Rat Cell Line Containing a Lambda Shuttle Vector for *In Vitro* Mutagenicity Testing. *Mutation Research* 334:161-165.
69. Snead, M.A., Kretz, P.L., and Short, J.M. (1995) Methods for Generating Plant Genomic Libraries. *Plant Molecular Biology Manual*, Kluwer Academic Publishers: Belgium (H1, 1-19).
70. Robertson, D.E., Mathur, E.J., Swanson, R.V., Marrs, B.L., and Short, J.M. (1996) The Discovery of New Biocatalysts From Microbial Diversity. *SIM News* 46:3-8.
71. Knoll, A., Jacobson, D.P., Nishino, H., Kretz, P.L., Short, J.M., and Sommer, S. (1996) A Selectable System for Mutation Detection in the Big Blue® /lacI Transgenic Mouse System: What Happens to the Mutational Spectrum Over Time. *Mutation Research* 352:9-22.
72. Wyborski, D.L., DuCoeur, L.C., and Short, J.M. (1996) The Effect of Chromosome Position and Operator Placement on Lac Repressor Control in Eukaryotic Cells and Transgenic Mice. *Environmental and Molecular Mutagenesis*. 28:447-458.
73. Sick, A.J., Fernandez J. and Short, J.M. (1996) Multiple Purpose Cloning Vectors. *Molecular Biology* (published).
74. Snead, M., Alting-Mees, M.A., and Short, J.M. (1997) cDNA Library Construction for the Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, cDNA Library Protocols*. Humana Press 69:39-51.
75. Snead, M., Alting-Mees, M.A., and Short, J.M. (1997) Clone Excision Methods for the Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, cDNA Library Protocols*. Humana Press 69:53-60.
76. Short, J.M. (1997) Recombinant Approaches for Accessing Biodiversity. *Nature Biotechnology* 15:1322-1323.
77. Nichols, W.S., Geller, S.A., Edmond, V.J., Dyaico, M.J., Sorge, J.A., and Short, J.M. (1998) Hepatocarcinogenesis (Z#2) / mutagenesis during initiation stage. *Mutation Research* 398:143-149.
78. Snead, M., Alting-Mees, M.A., and Short, J.M. (1998) cDNA Library Construction for Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, Plant Virology Protocols*. Humana Press 81:255-267.
79. Bruggeman, T., Short, J.M., and Simms, P. (1998) Diversa: Catalyzing a Revolution. *Industrial Biotech News* 1(1):4,14-15.
80. Deckert, G., Warren, P.V., Gaasterland, T., Young, W.G., Lenox, A.L., Graham, D.E., Overbeek, R., Snead, M., Keller, M., Aujay, M., Huber, R., Feldman, R.A., Short, J.M., Olsen, G.J., and Swanson, R.V. (1998) The Complete Genome of the Hyperthermophilic Bacterium *Aquifex aeolicus*. *Nature* 392:353-358.
81. Li, J., Robertson, D.E., Short, J.M., Wang, P.G. (1999) Chemical and enzymatic synthesis of glycoconjugates. 5: One-pot regioselective synthesis of bioactive galactobiosides using a CLONEZYME thermophilic glycosidase library. *Bioorganic & Medicinal Chemistry Letters Jan 4; 9 (1):35-8*

82. Snead, M.A., Alting-Mees, M.A., Short, J.M. (2000) cDNA Library Construction for the Lambda ZAP® - Based Vectors. *Nucleic Acid Protocols Handbook*, Humana Press Part V:355-365.
83. Sehgal, A.C., Callen W., Mathur E. J., Short, J.M., Kelly, R.M. (2001) Carboxylesterase from *Sulfolobus Solfataricus* P1. *Methods in Enzymology* 330:461-471.
84. Cady, S.G., Bauer, M.W., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Beta-Endoglucanase from *Pyrococcus Furiosus*. *Methods in Enzymology* 330:346-354.
85. Miller, E.S., Kimberley, Parker, N., Liebl, W., Lam, D., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Alpha-D-galactosidases from *Thermotoga* Species. *Methods in Enzymology* 330:246-260.
86. Chhabra, S., Parker, K.N., Lam, D., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Beta-mannanases from *Thermotoga* Species. *Methods in Enzymology* 330:224-238.
87. Parker, K.N., Chhabra, S.R., Lam, D., Callen, W., Duffaud, G.D., Snead, M.A., Short, J.M., Mathur, E.J., Kelly, R.M. (2001) Galactomannanases Man2 and Man5 from *Thermotoga* species: growth physiology on galactomannans, gene sequence analysis, and biochemical properties of recombinant enzymes. *Biotechnology and Bioengineering* Nov 5;75 (3):322-33
88. Gray, K.A., Richardson, T.H., Kretz, K., Short, J.M., Bartnek, F., Knowles, R., Kan, L., Swanson, P.E., Robertson, D.E. (2001) Rapid Evolution of Reversible Denaturation and Elevated Melting Temperature in a Microbial Haloalkane Dehalogenase. *Advanced Synthesis & Catalysis* 2001, 343:607-617.
89. Richardson, T.H., Tan, X., Frey, G., Callen, W., Cabell, M., Lam, D., Macomber, J., Short, J.M., Robertson, D., Miller, C. (2002) A Novel, High Performance Enzyme for Starch Liquefaction: Discovery and Optimization of a Low pH, Thermostable α -amylase. *Journal of Biological Chemistry* 2002, 277(29), 26501-26507.
90. Zengler, K., Toledo, G., Rappe, M., Elkins, J., Mathur, E.J., Short, J.M., Keller, M. (2002) Cultivating the Uncultured. *Proceedings of the National Academy of Sciences of the United States of America* (2002), 99(24), 15681-15686.
91. Murphy, K.M., Broman, K.W., Ziegler, J.S., Wyborski, D.L., Joe, L.K., Smith, D.W., Thurston, L.M., Stevenson, S.E., McClelland, M., Short, J.M., Mathur, E.J., Varley, J.D. (2002) Successful Breeding Among Free-Ranging 10-Month-Old Gray Wolves (*Canis Lupus*) in Yellowstone National Park, Wyoming. *Yellowstone Center for Resources* (2002). Kerry M. Murphy (kerry_murphy@nps.gov), P.O. Box 168, Yellowstone National Park, WY 82190.